Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 7236–7241, July 1996
Medical Sciences

Increased liposome extravasation in selected tissues: Effect of substance P

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Communicated by Richard J. Havel, University of California, San Francisco, CA, March 26, 1996 (received for review July 3, 1995)

ABSTRACT **We have used a pharmacologic mediator to open intercellular connections in selected vessels to allow liposomes to escape from the blood stream and to extravasate into tissues that have appropriate receptors. We have examined the effects of substance P (SP), a peptide known to increase vascular permeability in selected tissues, such as trachea, esophagus, and urinary bladder in rats. We used quantitative fluorescence analysis of tissues to measure two fluorescent markers, one attached to the lipid (rhodamine-phosphatidylethanolamine) and another, doxorubicin (an anti-tumor drug), encapsulated within the aqueous interior. We have also examined the deposition of liposomes microscopically by the use of encapsulated colloidal gold and silver enhancement. Analysis of the biochemical and morphological observations indicate the following: (*i*) Injection of SP produces a striking increase in both liposome labels, but only in tissues that possess receptors for SP in postcapillary venules; (*ii*) liposome material in these tissues has extravasated and is found extracellularly near a variety of cells beyond the endothelial layer over the first few hours; (*iii*) 24 h following injection of liposomes and SP, liposome material is found in these tissues, localized intracellularly in both endothelial cells and macrophages. We propose that appropriate application of tissue-specific mediators can result in liposome extravasation deep within tissues that normally do not take up significant amounts of liposomes from the blood. Such liposomes are able to carry a variety of pharmacological agents that can be released locally within selected target tissues for therapeutic purposes.**

Liposomes have been used as carriers for drugs and macromolecules (1, 2), as carriers for DNA to transfect cells *in vitro* (3), and to deliver genes into specific tissues for gene therapy (4, 5). However, the ability of liposomes administered via the bloodstream to reach target cells in various tissues is severely limited by two factors. First, conventional liposomes are cleared rapidly from the circulation by the phagocytic cells of the reticuloendothelial system (6). Thus, following i.v. injection, the majority of conventional liposomes are taken up by liver and spleen within a few minutes to hours, depending on particle size and lipid composition (6, 7). Second, most tissues have an endothelial barrier that does not permit particles of the size of even the smallest liposomes to extravasate (8). The recent development of sterically stabilized liposomes, which have a markedly prolonged residence time in blood (9, 10), has made it possible to deliver liposome-encapsulated drugs to tumors (10–14) and to sites of infection (15, 16) that have increased endothelial permeability to such particles.

An alternative possibility for delivering liposomes to selected tissues is to use a pharmacologic mediator that, by opening intercellular connections in selected vessels, might allow liposomes to escape from the bloodstream and extravasate into the tissues of organs whose postcapillary endothelia contain receptors for the mediator (17, 18). In the present study, we examined the effects of substance P (SP), a peptide that increases vascular permeability only in selected tissues. First, we studied the ability of SP to allow liposomes to escape from the bloodstream and extravasate into the tissues of organs that contain SP receptors (such as trachea, esophagus, and urinary bladder) using rhodamine-phosphatidylethanolamine (PE) as a liposomal marker. Second, we investigated the ability of SP to increase the tissue uptake of a liposome-encapsulated drug ito examine whether the extravasated liposomes can retain their contents for possible pharmacological actions. As a model drug we have chosen doxorubicin, an anti-tumor agent that can be encapsulated with high efficiency within the interior aqueous space of the liposomes. Finally, we studied the anatomic localization of liposomes in tissues using microscopic imaging of encapsulated colloidal gold-containing liposomes. Our findings indicate that the use of specific mediators of inflammation, such as SP, can produce a large increase in the extravasation of liposomes along with their encapsulated contents into specific tissues. These results appear to have general applicability, because liposomes have been used to successfully encapsulate of pharmacological agents including beta-adrenergic antagonists, antibiotics, oligonucleotides, and DNA (1, 2).

MATERIALS AND METHODS

Materials. Hydrogenated soy phosphatidylcholine was purchased from Natterman (Cologne, Germany). Distearoylphosphatidylethanolamine derivatized at the amino position with a segment of polyethylene glycol-PE (molecular weight, 1900) was synthesized as described (9) and was obtained from Sequus Pharmaceuticals (Menlo Park, CA). EggPC, dioleoylphosphatidylethanolamine (DOPE), and *N*-(lissamine rhodamine B sulfonyl) diacyl phosphatidylethanolamine were purchased from Avanti Polar Lipids. Highly purified cholesterol was purchased from Calbiochem. Deferoxamine mesylate (desferal) and dimethyl-dioctadecylammonium bromide (DDAB) were obtained from Sigma. Doxorubicin hydrochloride was obtained from Chiron. SP, purity 98% (HPLC), was purchased from Bachem. Other solvents and chemicals were of analytical grade.

Preparation of Liposomes. Sterically stabilized liposomes composed of hydrogenated soy phosphatidylcholine/cholesterol/polyethylene glycol-PE/*N*-(lissamine rhodamine B sulfonyl) diacyl phosphatidylethanolamine (molar ratio, 30:20:1.8:0.24) were prepared by hydration of thin lipid films with Hepes·NaCl buffer (20 mM Hepes/144 mM NaCl, pH 7.2,

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Abbreviations: SP, substance P; PE, phosphatidylethanolamine.

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300 mosmol) at 57°C. The hydrated lipid suspension was put through freezing (dry ice and ethanol) and thawing (57°C) cycles five times. The multilamellar liposomes thus formed were extruded at 57°C under argon through polycarbonate membranes, through 0.1 μm pore size three times, and then through 0.05 μm pore size five times (19). Then the liposome solution was put through cold water (≈20°C) and hot water (57°C) cycles three times, followed by passage through a Sephadex G-75 column equilibrated with Hepes·NaCl buffer.

Doxorubicin-loaded liposomes contained the same lipid components and molar ratio as the liposomes described above, but no rhodamine-PE. The dry lipid mixture was hydrated with ammonium sulfate solution (250 mM $(NH_4)_2SO_4$/1 mM desferal), followed by freeze-thaw cycles, extrusion, cold-hot water cycles, and a Sephadex G-75 column, as described above. Doxorubicin (1 mg per 10 μmol of phospholipid) was loaded into liposomes by dissolving doxorubicin dry powder in the liposome solution and shaking in a water bath at 57°C for 30 min. (20). Free doxorubicin was then removed on a Sephadex G-75 column. The final preparation contained 0.1 mg of doxorubicin per μmol of phospholipid.

Liposomes containing colloidal gold were prepared according to the published procedure (21). Liposomes composed of EggPC/cholesterol/polyethylene glycol-PE (30:20:1.8) were prepared by reverse-phase evaporation in gold chloride solution (6.36 mM $HAuCl_4$/60 mM citric acid/15 mM K_2CO_3, pH 3.5) at a concentration of phospholipid of 10 μmol/ml. The liposomes obtained from reverse-phase evaporation were extruded through polycarbonate membrane filters (19), 0.1 μm pore size (twice), and 0.05 μm pore size (five times) to obtain liposomes with a narrow size distribution. Immediately after the final extrusion, the colloidal gold formation was promoted by raising the pH of the liposome suspension to 5.5–6.0 by adding NaOH and incubating at 55°C for at least 30 min. The unencapsulated gold particles were separated from gold liposomes by passing the liposome suspension through a Sephacryl S-400 column (Pharmacia). The gold-containing liposomes were characterized by microscopy (21) and were stable at 4°C under argon for at least 1 month.

***In Vivo* Procedures.** Experimental procedures followed in this study were approved by the Committee on Animal Research of the University of California, San Francisco. We used pathogen-free male rats of the F344 strain, which were 10–12 weeks old, 250–300 g, and obtained from Simonsen Laboratories (Gilroy, CA). Three different types of liposomes were used to examine the deposition of liposomes in various tissues.

Experiments with rhodamine-labeled liposomes (three groups of rats). In one group of rats ($n = 4$), rhodamine-PE labeled liposomes (15 μmol of phospholipid per kg of body weight) were injected into a femoral vein. SP (10 nmol/kg) or 0.9% saline was injected i.v. 5 min later. After another 5 min, rats were perfused with PBS as follows. A cannula was inserted into the ascending aorta through the left ventricle, the left atrium was incised, and perfusion was carried out for 1 min. Then the right atrium was cut and perfusion was continued for another 2 min. In a second group of rats ($n = 5$), injection of SP was repeated three times at 5, 35, and 65 min after injection of liposomes, to evaluate the effect of multiple injections of SP. In a third group of rats ($n = 3$), 0.3 ml of blood samples was taken from a femoral artery immediately before and 10 min after the injection of liposomes for measurement of liposome concentration over time.

Experiments with doxorubicin-encapsulated liposomes. In one group of rats ($n = 5$), doxorubicin-loaded liposomes (15 μmol of phospholipid per kg of body weight) were injected into a femoral vein. SP (10 nmol/kg) or saline was injected i.v. 5 min later. After another 5 min, the rats were perfused as described above. In a second group of rats ($n = 3$), 0.3 ml of blood samples was taken from a femoral artery at 1, 10, 30, and 60 min after liposome injection. These studies were performed to determine the concentration of doxorubicin-loaded liposomes in the blood over time.

Experiments with liposomes containing colloidal gold. We injected liposomes containing colloidal gold (15 μmol of phospholipid per kg of body weight; $n = 4$) into a femoral vein, and 5 min later we injected SP (10 nmol/kg) or the vehicle control without SP (0.9% saline). The rats were euthanized and perfused at varying intervals (5 min, 60 min, 6 h, or 24 h) after SP was administered.

Tissue Analysis of Rhodamine-Labeled Lipid and Doxorubicin. The fluorescence intensity of rhodamine was measured after lipids were extracted from tissues. Tissues were first homogenized in 50% methanol, and then the lipids were extracted with chloroform with a volume ratio of 1:1:5.3 (methanol/water/chloroform). Extraction was carried out by shaking for ≈15 h at room temperature. Fluorescence signals were measured using Soex Fluorolog (Edison, NJ) with excitation slits of 1.25 mm and emission slits of 5 mm. Rhodamine in chloroform was excited at 560 nm, and emission was observed at 595 nm.

To measure doxorubicin, tissues were homogenized with 50% ethanol and then mixed with an equal volume of 50% ethanol containing 0.6 M HCl, followed by shaking for ≈15 h at room temperature. In this way, doxorubicin was released from liposomes. Fluorescence measurements were obtained using the same equipment and slits as described above. Doxorubicin was excited at 470 nm, and emission peaks occurred at 555 nm and 590 nm. The fluorescence intensity at 555 nm and 590 nm was converted to the amount of doxorubicin in the tissue by comparing values to a standard curve of doxorubicin. Rats that were only injected with saline provided background fluorescence signals for the measurements of rhodamine and doxorubicin.

Blood samples containing liposomes were treated as follows. We added 0.75 ml of PBS and centrifuged the blood to separate the plasma from red cells. Then we added 0.75 ml of PBS to the cell pellet and centrifuged again. Both supernatants were combined, and rhodamine-PE was extracted with 4 ml of chloroform, and doxorubicin was extracted with ethanol containing 0.6 M HCl.

Light Microscopy Following Silver Enhancement of Colloidal Gold. The tissues were fixed in 3% glutaraldehyde/1% paraformaldehyde overnight at 4°C. The tissues were then rinsed with buffer at 4°C for 30 min and dehydrated with acetone for 15 min each in 70% acetone, 2% dimethyl sulfoxide/95% acetone, 2% dimethyl sulfoxide, and 100% acetone. Following dehydration, the tissues were embedded in JB4 resin obtained from Polysciences, allowed to polymerize, and cut (2.5-μm sections) on a Reichert JB4 microtome. The tissues containing liposome–gold complex were then incubated for 15 min with silver enhancement reagents (Amersham). The sections were counterstained with hematoxylin and examined.

Statistical Analysis. All data are expressed as mean ± SEM. Statistical analysis was performed using a Macintosh computer and the STATVIEW program. Analysis of variance was performed for quantitative data by the Student's t test for unpaired samples. Differences were considered statistically significant when $P < 0.05$.

RESULTS

Localization of Liposomes Labeled with Rhodamine-PE. To study the effect of SP on deposition of liposomes in various tissues, sterically stabilized liposomes were labeled with rhodamine-PE, a lipid-soluble fluorescent probe. SP or saline was introduced into rats 5 min after injection of liposomes. The animals were perfused 5 min later, and rhodamine fluorescence from 10 different tissues was measured.

In the trachea, esophagus, and urinary bladder, the liposome lipid concentration was low in the absence of SP, but the

concentration of liposome marker increased markedly after SP (Fig. 1). Thus, the amount of liposome lipid after SP averaged 24 times greater than control in trachea, 55 times greater in esophagus, and four times greater in urinary bladder. In other tissues, including ileum, skin, kidney, heart, and lung, and in the absence of SP, liposome concentrations were also low, and in these tissues liposome concentrations were also unaffected by SP. In the liver and spleen, liposome concentrations were higher than in other tissues in the control state, but these amounts were also unaffected by SP (Fig. 1).

We also examined the rhodamine fluorescence of blood samples after injection of rhodamine-PE-labeled liposomes to determine whether the concentration of the liposomes in the bloodstream changed over time during the experiments. Ten minutes after injection of liposomes, ≈100% of the original amount of the liposomes remained in the blood ($n = 3$; data not shown). In another experiment, introduction of SP was repeated three times (5, 35, and 65 min after i.v. injection of liposomes). The uptake of phospholipid was not greater in trachea, esophagus, and bladder than in the animals given only one injection of SP (data not shown).

Localization of Doxorubicin Encapsulated in Liposomes. To determine whether liposome contents are retained within the liposomes during extravasation, we also encapsulated doxorubicin (an anti-tumor drug) into the aqueous interior of liposomes. Doxorubicin-loaded liposomes were injected i.v. into rats, followed by the injection of SP or saline 5 min later. Five minutes after SP or saline, the animals were perfused, and doxorubicin fluorescence was measured in 10 tissues. In blood, the concentration of doxorubicin was unchanged during the experimental procedure ($n = 3$; data not shown). The findings in trachea, esophagus, and urinary bladder were similar to the results with rhodamine-labeled liposomes. In the control state without SP, doxorubicin concentrations were uniformly low; after SP, doxorubicin increased markedly in all three tissues (Fig. 2), with doxorubicin concentrations increased 52-fold in trachea, 138-fold in esophagus, and 13-fold in urinary bladder. On the other hand, the concentrations of doxorubicin in ileum, skin, kidney, heart, liver, and lung were not affected by SP. In the spleen, the doxorubicin concentration was high in the control state but unaffected by SP (Fig. 2).

Localization of Gold-Labeled Liposomes. We performed morphological studies with gold-labeled, sterically stabilized liposomes. The presence of colloidal gold particles is visualized by silver enhancement and is taken as evidence for the presence of liposomes (11, 13). In the tracheas of rats that received no SP, the colloidal gold/silver particles were visualized only within the lumen of the blood vessels; no liposomes were found in the tracheal tissue (Fig. 3*a*). In rats that received liposomes and then SP 5 min later, the gold label was dispersed diffusely throughout the submucosal tissue between the cartilage and the epithelium, with the highest concentration of gold surrounding the small venules. Except in occasional small foci, the epithelium itself remained free of liposomes (Fig. 3*a*). The pattern of dispersion of gold particles after SP in the esophagus (Fig. 3*b*) and in the urinary bladder (Fig. 3*c*) was similar to the trachea. In all other tissues studied, the liposomes remained only in the vascular lumens, even after SP.

To determine the time sequence for distribution and clearance of gold particles after extravasation, a group of rats were euthanized at varying time intervals after the i.v. injection of the gold-labeled liposomes. Five minutes after administration of SP, the gold label was dispersed diffusely in tracheal tissue (Fig. 4, *Middle*). At 60 min after administration of SP, gold particles were dispersed to approximately the same degree within the trachea as at 5 min (data not shown). However, at

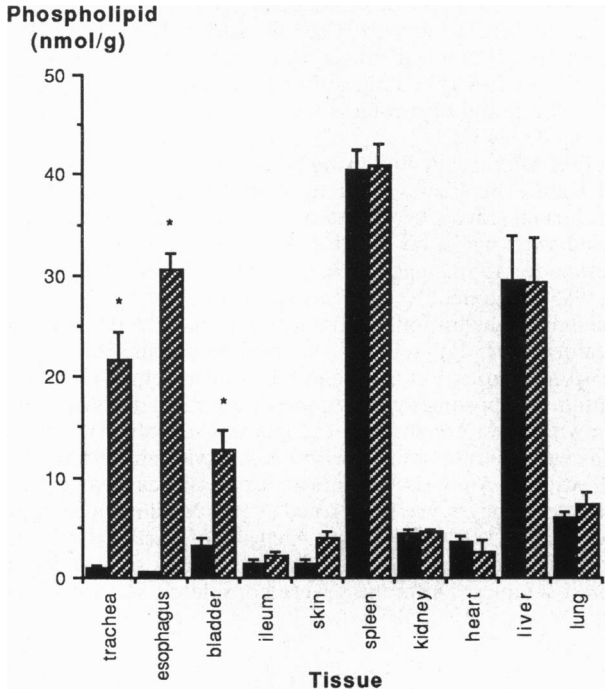

FIG. 1. Effect of SP on the deposition of sterically stabilized liposomes labeled with rhodamine-PE in various tissues of rats. Liposomes (15 μmol of phospholipid per kg of body weight) were injected i.v., and 5 min later either the vehicle control without SP (0.9% saline; solid columns; $n = 4$) or SP (10 nmol/kg; hatched columns; $n = 4$) was injected. Animals were euthanized 10 min after liposome injection, and the circulation was perfused with PBS to remove the blood. Liposome concentrations in tissues are expressed as phospholipid in nmol per g of wet tissue. Data reported as mean ± SEM. *, significantly different from controls ($P < 0.05$).

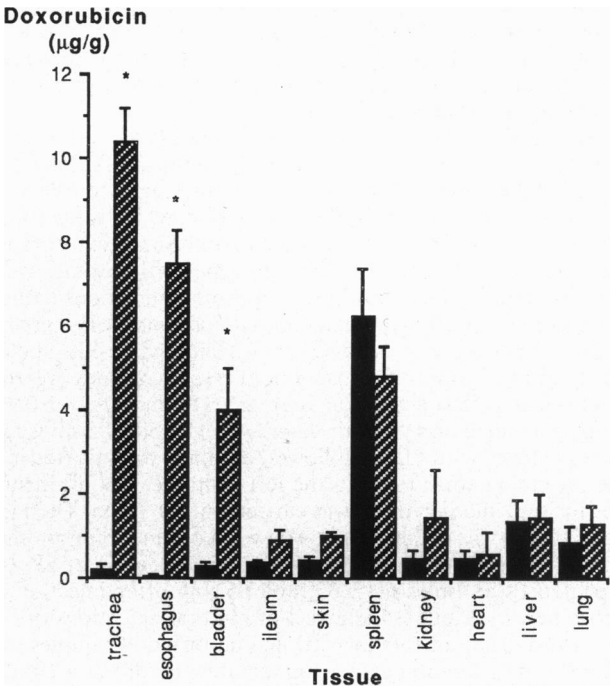

FIG. 2. Effect of SP on the deposition of sterically stabilized liposomes loaded with doxorubicin in various tissues in rats. Liposomes (15 μmol phospholipid per kg of body weight) were injected i.v., and 5 min later either the vehicle control without SP (0.9% saline; solid columns; $n = 5$) or SP (10 nmol/kg; hatched columns; $n = 5$) was injected i.v. Animals were euthanized 10 min after liposome injection, and the circulation was perfused with PBS to remove the blood. Doxorubicin concentrations were measured and expressed as μg per g of wet tissue. Data reported as mean ± SEM. *, significantly different from controls ($P < 0.05$).

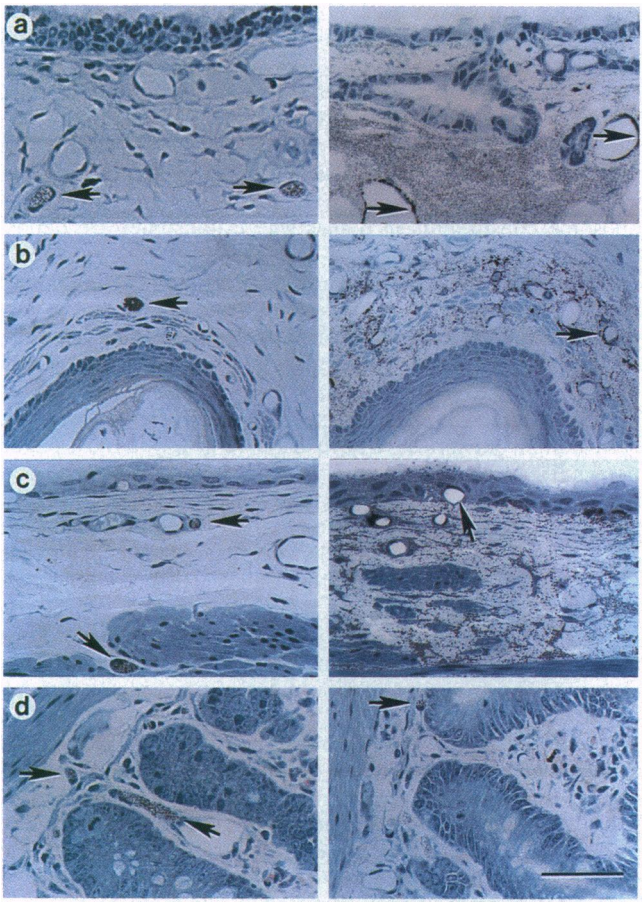

FIG. 3. Effect of SP on the localization of gold-labeled sterically stabilized liposomes in various tissues of rats: *a*, Trachea; *b*, esophagus; *c*, urinary bladder; and *d*, ileum. Animals were euthanized 10 min after liposome injection. When no SP is administered (*Left*), the gold/silver particles representing liposomes are located only in the vascular lumen. In rats given liposomes and then SP 5 min later (*Right*), the particles are dispersed diffusely throughout the submucosal tissue in the trachea, esophagus, and urinary bladder, with the highest concentration of particles surrounding small vessels. Except for occasional small foci, the epithelium remains free of particles. In the ileum, the particles remain in the vascular lumens, even after SP. Staining was performed with hematoxylin. Scale bar = 25 μm. Arrows indicate blood vessels.

6 h, there was less widespread dispersion of the gold particles; many particles were localized intracellularly in endothelial cells. This disappearance of widely dispersed particles and their localization in small blood vessels was more evident in the tissue at 24 h (shown at low power in Fig. 4, *Lower*).

In the liver, liposomes were seen intracellularly within the macrophage cells lining the sinusoids, both in control rats and in rats given SP. No difference in the uptake of liposomes was noted between controls and SP-injected rats (data not shown).

DISCUSSION

The purpose of the present study was to examine whether liposomes could be made to "home" into selected tissues by causing an increase in their vascular permeability. We injected SP, a neuropeptide that is known to cause vascular extravasation, in selected tissues whose postcapillary venules possess SP receptors (e.g., trachea, esophagus, and urinary bladder) but not in organs whose postcapillary venules do not possess SP receptors (e.g., ileum, heart, and kidney; refs. 17 and 22). Our results indicate that SP causes striking increases in the tissue concentrations of both rhodamine-PE and doxorubicin in trachea, esophagus, and urinary bladder, but not in other

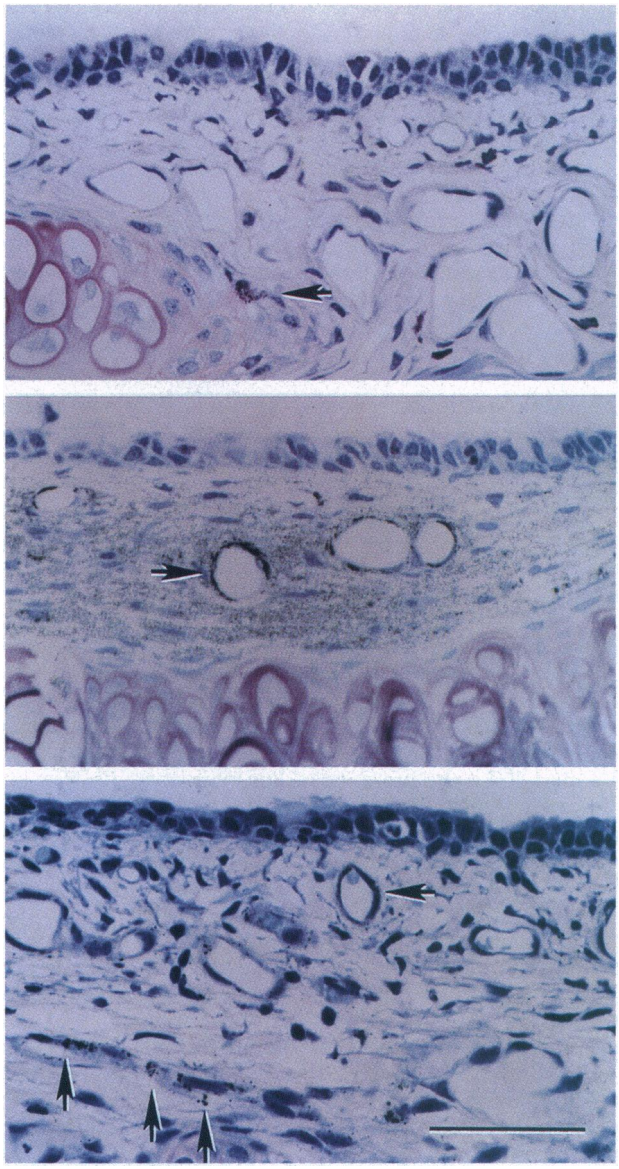

FIG. 4. Time-dependent effect of extravasation and clearance of gold-labeled sterically stabilized liposomes in the trachea of rats. (*Upper*) No SP. The gold/silver particles remain within the vessels. (*Middle*) Five minutes after SP administration, the particles are dispersed diffusely throughout the submucosal tissue. (*Lower*) At 24 h, many of the gold/silver particles are seen in the walls of small blood vessels. Staining was performed with hematoxylin. Bar = 25 μm. Arrows indicate blood vessels.

tissues. These results are impressive, especially considering the short period of time that SP is capable of increasing extravasation (22). In fact, we used SP to increase vascular permeability to test whether very short periods of extravasation could allow selective deposition of liposomes in tissues. It is known that when SP is injected i.v., extravasation in the trachea occurs only for a short time (half-time, 2.4 min; ref. 23). Thereafter, there is long-lasting desensitization to SP. Other studies suggest that the rapid desensitization to SP is due, at least in part, to rapid internalization of SP receptors (24). In the airways, internalization of NK1 receptors is believed to play a role in limiting the duration of SP-induced extravasation (25).

It has been recognized for some time that various mediators of inflammation (e.g., histamine and SP) cause increased vascular permeability by opening gaps in the postcapillary venular endothelium (26); this allows plasma proteins and other macromolecules (either resident in the blood or injected

into the circulation as "markers") to escape into the extravascular tissue. One such marker is Evans blue dye, which binds spontaneously to plasma albumin and thus tests the permeability of blood vessels to molecules of ≈60 kDa. When vascular permeability is increased, the dye extravasates into the extravascular tissue and moves rapidly through the connective tissue after extravasation. Particulate tracers of much larger dimensions, such as Monastral blue (greatest dimension, ≈300 nm) have also been injected into the bloodstream (27). Monastral blue normally remains in the circulation and is cleared slowly by the reticuloendothelial system. However, after injection of SP, the particles of dye leak through the endothelium in organs that contain SP receptors. These large particles are reported to remain trapped in the endothelial basal lamina (27).

Sterically stabilized liposomes (diameter, ≈100 nm) remain in the peripheral blood for long periods of time (10) and uptake by normal tissues is limited, except for tissues with resident macrophage cells such as the liver and spleen (10). Other tissues reported to accumulate long-circulating liposomes are tumors (11–14) and *Klebsiella pneumoniae*-infected lung tissue (15, 16). In the present study, we examined the "homing" of liposomes to selected tissues by increasing vascular permeability selectively. For quantitative estimation of liposome deposition in tissues, we used fluorescence spectroscopy, using two different fluorescent labels: rhodamine attached covalently to phosphatidylethanolamine was used as a marker for the uptake of lipid material, and doxorubicin encapsulated into the aqueous interior of liposomes was used as a marker for the presence of liposome contents. Previous studies have shown that doxorubicin can be stably encapsulated in sterically stabilized liposomes, with a half-life in blood of 14–18 h in rodents (10). Its presence in various tissues is an indication for the uptake of liposomes still containing at least some of their encapsulating contents, as has been seen previously in some tumors implanted in mice (11–14). The biochemical markers allowed us to show that SP permits liposomes to leave the bloodstream and to enter selected tissues. We performed morphological studies using liposome-encapsulated colloidal gold to examine the anatomic localization of liposomes in tissues. Previous studies showed that 24 h after i.v. injection of gold-containing liposomes in mice, the ratio of gold-containing to unlabeled liposomes in plasma was unchanged, suggesting that the gold particles in tissue represent intact liposomes (11). Furthermore, electron microscopy has established the presence of intact liposomes, still encapsulating gold, within tissues in mice after 24 h. (13). In the present studies, we used silver enhancement of colloidal gold to localize liposomes in hematoxylin-stained sections of various tissues. Using this technique, silver-coated gold particles could be visualized as black dots by regular light microscopy within the lumens of blood vessels or within tissue, either extracellularly or intracellularly (Figs. 3 and 4).

Analysis of the biochemical and morphological observations described above indicate the following points. (*i*) SP produces a large effect in the tissues whose postcapillary venules possess receptors for SP (trachea, esophagus, and urinary bladder) where the uptake of liposome material per g of tissue weight is strikingly increased after SP, whereas the uptake observed in liver and spleen, (the organs that are normally the main recipients for liposome uptake) is unaffected by SP. (*ii*) In tissues containing SP receptors, extravasation of liposomes is localized in the extracellular spaces of these tissues beyond the postcapillary venular endothelium. In contrast, the uptake in the liver is intracellular, within the macrophage cells lining the sinusoids. (*iii*) Extravasated liposome material in SP-sensitive tissues extends to the vicinity of a variety of cells well beyond the endothelial layer. Thus, in the trachea, the early deposition of liposomes extends from the border of the cartilage to the tissue immediately beneath the epithelium. However, very little uptake of gold particles by epithelial cells was observed. (*iv*) Over a period of many hours after SP, most of the gold particles eventually appear to become concentrated intracellularly, both in tissue macrophages and in endothelial cells. However, it is not certain whether the intracellular gold particles remain encapsulated or released from liposomes at these later time periods. Further studies are required to investigate longterm changes in concentrations of various liposome constituents in tissues where liposomes are deposited.

From the biochemical and morphological findings described above, we conclude that sterically stabilized liposomes, which normally remain in the bloodstream for long periods of time, can be taken up by selected tissues following the injection of substances that modify vascular permeability. In contrast to Monastral blue (23), the liposomes are not confined to the basal lamina but appear to be distributed widely in the affected tissues. The exact mechanisms responsible for the dispersion of particles in the tissues following extravasation are unknown. In addition to physical dimensions, their movement may be related to physicochemical characteristics, such as surface charge and hydrophilicity. After crossing the endothelium, movement beyond the basal lamina cannot be completely limited by physical dimensions, because neutrophils normally migrate across the endothelium and through the tissue without restraint. The dynamics of movement of particulates through tissue is an important, largely unexamined phenomenon.

As shown by the present biochemical and morphological findings, organs such as liver (Kupfer cells), which normally take up liposomes show no change in liposome uptake after SP. Morphologic studies in the liver before and after SP showed that liposomes were only found in the phagocytic cells and within the vascular lumens; none were found adjacent to hepatocytes.

In this study, we have used SP to produce extravasation of liposomes and to increase the deposition in selected tissues. Studies of extravasation using different mediators of inflammation (e.g., histamine, serotonin, and bradykinin) indicate that extravasation sites can be selected by the choice of mediator (22). In the present study, we injected the mediator (SP) i.v., which may have undesirable side effects. We envision the use of the extravasation-enhancing mediator in anatomically restricted areas, such as into the urinary bladder following instillation via a catheter or into airways following inhalation. In such cases, extravasation can be localized to single tissues such as the trachea (28). In this way, the effect of the mediator in enhancing the uptake of circulating liposomes can be expressed in the specific tissues desired without systemic effects.

Our demonstration that the delivery of doxorubicin in the trachea, esophagus, and urinary bladder can be strikingly increased by SP is an example of enhanced local drug delivery, which could be applied to various therapies. In the future, this technique may be useful for such diverse therapies as the delivery of antiinflammatory drugs to selected postcapillary venules or the delivery of genes to selected tissues.

This work was supported in part by National Institutes of Health Program Project Grants HL24136 and Grant GM28117 and by National Institutes of Health Grant DK47766. J.R. was supported by a fellowship from the Cystic Fibrosis Research and Development Program and the German Cystic Fibrosis Foundation. W.Z. was funded by the National Institutes of Health Institutional Researh Service Award T32 HL07185, Multidisciplinary Research Training Program in Lung Disease.

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